3. (amended) The method of claim 1 where said animal cell culture is a mammalian cell culture.

6. (amended) The method of claim 4, wherein said monoclonal antibodies are human or murine monoconal antibodies.

REMARKS

Claim 2 has been cancelled, and claims 1, 3, and 6 have been amended. Specifically, for purposes of clarifying the invention, that limitation encompassed by claim 2 is now incorporated into claim 1, item (b). The preamble of claim 1 has been amended to clarify that the concentration of the solute of interest in the culture medium composition for optimal product expression is different that the concentration of this solute in the culture medium composition determined for optimal cell growth. Claim 1, item (a) has been amended to provide antecedent basis for the culture medium recited in claim 1, item (b). Claim 1, item (c) has been amended to clarify that product expression is monitored as concentration of the solute is varied in the culture medium. In view of cancellation of claim 2, claim 3 has been amended to depend from claim 1 instead of claim 2. Claim 6 has been amended to depend from claim 4 to provide proper antecedent basis for the phrase "monoclonal antibodies." Support for these amendments to the claims resides throughout the specification and in the original claims. No new matter is added by way of claim amendment.

The specification has been amended as noted herein below in order to address the Examiner's objections. No new matter is added by way of amendments to the specification.

Claims 1 and 3-6 are now pending in the application. The Examiner's comments in the Office Action are addressed below in the order set forth therein.

Submittal of Formal Drawing

Responsive to the request for correction of the informalities indicated on the Notice of Draftperson's Patent Drawing Review, PTO Form 948, Applicants submit concurrently herewith

a new formal drawing for Figure 1. The two views in this figure are now labeled Fig. 1A and Fig. 1B. The Examiner is respectfully requested to enter this formal drawing into the above-referenced application in place of the formal drawing for this figure as filed on October 18, 2001.

Objections to the Specification

The specification has been amended at pages 3, 9, 10, and 11 to spell out the first instance of the use of the term "mOsmol/kg", "ELISA," "LPS," and "rpm," respectively, and to correct obvious typographical errors at page 2 and page 6. No new matter has been added by way of amendment. In view of these amendments, Applicants respectfully submit that the objections to the specification should be withdrawn.

The Rejections of the Claims Under 35 U.S.C. §112, Second Paragraph, Should Be Withdrawn

Claims 1-6 are rejected under 35 U.S.C.§112, second paragraph, as being indefinite. This rejection is respectfully traversed as applied to the amended claims.

Claim 1, item (d) is rejected for recitation of the phrase "selecting the solute concentration that provides the optimal combination of cell growth and product expression," in view of claim 1, item (c), where at least two concentrations were determined. The Office Action states that it is unclear how the two concentrations are "the solute concentration" for optimal product expression, and points to the recitation of "selecting the solute concentrations" as support that the concentrations are plural (Office Action mailed September 30, 2002, at page 4, first paragraph).

Applicants respectfully note that the phrase "selecting the solute concentrations" does not appear in any of the items recited in claim 1 as originally presented, nor does this phrase appear in the dependent claims as originally presented. Rather, original independent claim 1 clearly recited the step of "selecting *the* solute *concentration* that provides the optimal combination of cell growth and product expression which allows for optimal productivity" (claim 1, item (d); emphasis added).

Amended claim 1 clearly sets forth the steps to be undertaken to determine the optimal level of product expression in accordance with the methods of the present invention. Thus, the

animal cell culture is grown in a culture medium that provides for optimal cell growth. Solute concentration is then varied in the culture medium until it is above the concentration that provides for optimal cell growth, at which point the concentration creates an environment of solute stress on the cell culture as expressed by an inhibitory effect on cell growth or cell density of the cell culture. Product expression is monitored as concentration of the solute is varied in the culture medium so that one can determine optimal product expression. One then selects the solute concentration that provides the optimal combination of cell growth and product expression, which allows for optimal productivity.

In view of these amendments and remarks, Applicants respectfully submit that this claim is definite, and this rejection should be withdrawn.

Claim 6 is rejected as being indefinite for recitation of the limitation "said monoclonal antibodies" without having proper antecedent basis for this phrase. Applicants respectfully note that claim 6 has been amended to depend from claim 4, which provides proper antecedent basis for this phrase. In view of this, the claim is now definite, and this rejection of the claim should be withdrawn.

The Rejection of the Claims Under 35 U.S.C.§102 Should Be Withdrawn

Claims 1-6 are rejected under 35 U.S.C.§102 as being anticipated by Rupp *et al.* (GB 2153830). This rejection is respectfully traversed as applied to the amended claims.

The pending claims are directed to a method for determining the optimal level of product expression in a cultured animal cell line, where the focus is on stressing the cells during their culture. Applicants respectfully submit that Rupp *et al.* do not seek to stress their cultured cells. To the contrary, their objective is to increase protein production without having an adverse impact on cell growth or cell viability. Thus, for example, Rupp *et al.* state that the hypertonic media of their invention "promote protein production without adversely affecting viability of the cells or their growth rates" (at page 2, lines 24-25; emphasis added). The emphasis in this cited reference is on retaining cell viability and rate of cell mitosis. See Example 1, at page 6, lines 30-32, where it is noted that addition of excess amino acids to the culture medium "does not alter

the viability or rate of cell mitosis of protein producing cells but does cause increased protein production." See also at page 6, lines 58-59, of this cited reference where it is noted that there was an apparent increase in hybridoma growth rate when cultured in the hypertonic medium of the Rupp *et al.* invention; and Example 2, at page 7, lines 25-27, of this cited reference where it is stated that "changing the osmolarity of the solution by addition of saline had no significant effect on the cell growth" (at page 7, lines 25-27; emphasis added). Rupp *et al.* never teach or even suggest that the medium has to be made sufficiently hypertonic such that stress is induced on the cells and growth or cell density is inhibited, the two conditions specifically required by the method of independent claim 1 and claims 3-6 directly or indirectly dependent thereon. Nowhere does the Rupp *et al.* reference propose or even suggest that one can determine the optimal level of product expression by systematically increasing the level of solute in the culture medium and monitoring product expression to determine the solute concentration at which cell growth becomes inhibited but product expression is at its highest.

The hypertonic medium of Rupp *et al.* is primarily useful for cells cultured in capsules containing permeable membranes through which nutrients in the medium are accessible (see at page 2, lines 14-19). The data in Example 2 and Table 4 on page 7 of this cited reference reflect no significant change in antibody synthesis when hypotonic, isotonic, or hypertonic cell cultures are compared. Rupp *et al.* state that changing the osmolarity by addition of NaCl had no effect on the cell growth. Further, the growth curves in Figure 4 of this cited reference are very similar at all three osmolarities and show no decline in cell density yet. Thus it is likely that solute stress had not been reached at 400 milliosmoles, the maximum osmolarity tested. This example does not constitute solute stress, and no consistent increase in antibody production was seen.

This is distinct from the solute stress described in the present specification on page 6, lines 14-15, wherein solute stress is stated to "produce[s] a growth inhibitory effect or reduced final cell density, that is, a growth rate or maximum cell density less than that determined for optimal growth." Also see the present application at page 7, line 21, where it is noted that "such changes negatively affect the growth of cultured cells." These represent but two passages out of many that show that the stress encompassed by Applicants' claimed invention negatively affects the cells, unlike the result seen with the culture medium contemplated by Rupp *et al.*

Furthermore, Rupp *et al.* do not demonstrate the dramatic effects on antibody production that have been achieved using Applicants' invention, which notably are achieved despite dramatic reduction in cell growth. Thus, Example 2 of Appplicants' specification shows that when 400 mOsmol/kg NaCl was added at the start of culturing D-234 cells, the antibody output was over twice that of the control 300 mOsmol/kg (see Table 1), but that maximum viable cell density was one half of the control. Example 4 of Applicants' specification shows that a 2- to 2 ½-fold increase in antibody production can be achieved by adding 400 to 459 mOsmol/kg NaCl (see Table 2), but that maximum viable cell density was three-fourths of the control. Example 5 of Applicants' specification shows that over twice the antibody can be produced by adding between 40 and 60 mM lactate at the beginning of cell culture (see Table 3) but that a critical drop in cell densities occurred at this level. Example 6 and Figure 2 of Applicants' specification show that 10 mM NH_4Cl caused the cell culture to produce twice the antibody of the control, but the cells were negatively affected by its addition. Example 7 of Applicants' specification shows that antibody production is higher when more glucose is added, and that the cells were negatively affected. Example 8 of Applicants' specification shows that antibody production is higher when 8 μg/l polypropylene glycol is added and some negative effects were seen in the cell culture. Rupp *et al.* do not show such an increase in antibody production, and they do not show "an inhibitory effect on cell growth or cell density" as required by independent claim 1 and claims directly or indirectly dependent thereon.

In view of these remarks, Applicants respectfully submit that the Rupp *et al.* reference does not teach every element of Applicants' claimed invention, and thus is not an anticipatory reference. Therefore, this rejection of the claims should be withdrawn.

The Obviousness Type Double-Patenting Rejection Should Be Withdrawn

Claims 1-6 are rejected under the judicially created doctrine of the obviousness-type double patenting as being unpatentable over the claims of U.S. Patent No. 6,238,891. A terminal disclaimer in compliance with 37 C.F.R. §1.321(c) is filed concurrently herewith. In view of this submission, Applicants respectfully submit that this rejection is now overcome.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully submit that the objections to the drawings and specification, the rejections of the claims under 35 U.S.C. §§102 and 112, and the obviousness-type double-patenting rejection are now overcome and that this application is now in condition for allowance. Early notice to this effect is solicited. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject Application, the Examiner is invited to call the undersigned.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Commissioner For Patents, Washington, DC 20231.

Lynda-Jo Pixley

Lynda-Jo Pixley

Version with Markings to Show Changes Made:

In the Specification:

Please amend the paragraph at page 2, lines 20-24, to read as follows:

Efforts to increase antibody yield have focused primarily on means to optimize cell growth and cell density. The optimal conditions for cell growth of mammalian cell culture are generally within narrow ranges for each of the parameters discussed above. For example, typical culture conditions for mammalian hybridoma cell culture use a basal culture medium supplemented with nutritional additives, pH in the range of 6.8 to 7.4 at [35-37C]35-37°C.

Please amend the paragraph beginning at page 3, line 27, continuing through page 4, line 1, to read as follows:

Media osmolality for mammalian cell culture is usually held in the range of 280-300 (Jakoby, W.B., and Pastan, I.H., Methods in Enzymology, vol. LVIII, "Cell Culture", Academic Press (1979), pp. 136-137). Of course, the optimal value may depend upon the specific cell type. For example, as reported in Tissue Culture, Methods and Applications, edited by Kruse, Jr., P.F. and Patterson, Jr., M.K., Academic Press (1973) p. 704, human lymphocytes survive best at low (about 230 [mOsmol/kg]milliosmole/kg (mOsmol/kg)), and granulocytes at higher osmolalities (about 330 mOsmol/kg.) Mouse and rabbit eggs develop optimally in vivo at around 270 mOsmol/kg, 250-280 mOsmol/kg being satisfactory, while above 280 mOsmol/kg development is retarded. Iscove reports 280 mOsmol/kg to be optimum for growth of murine lymphocytes and hemopoietic cells, and Iscoves modified DME is adjusted for this growth promoting osmolality (Iscove, N.N. (1984) Method for Serum-Free Culture of Neuronal and Lymphoid Cells, pp. 169-185, Alan R. Liss, ed., New York.

Please amend the paragraph at page 6, lines 19-24, to read as follows:

As used herein the term "osmolality" refers to the total osmotic activity contributed by ions and non-ionized molecules to a media solution. Osmolality, like molality, relates to weight of solvent (mOsmol/kg [H2O]H_20) while osmolarity, like molarity, relates to volume (mOsM/liter solution). Osmolality is one method used to monitor solute stress. Standard osmolality refers to the optimum range of clonal growth of mammalian cells which occurs at 290±30 mOsmol/kg.

Please amend the paragraph beginning at page 9, line 25, continuing through page 10, line 1, to read as follows:

The method of the invention has been shown to increase antibody titer regardless of the presence or absence of serum in the medium. The cell lines used in the present invention may be cell lines of diverse mammalian origin. Rat, mouse, hamster, primate and human embodiments are contemplated, with human and murine embodiments illustrated in the examples which follow. The antibodies may be of any class with IgM and IgG types being specifically exemplified herein. The human embodiments specifically exemplified herein are the products of triomas synthesized by somatic cell hybridization using a mouse x human parent hybrid cell line and Epstein-Barr virus (EBV)-transformed human peripheral blood lymphocytes (PBLs) or splenocytes from non-immunized volunteers or volunteers immunized with available Gram-negative bacterial vaccines or inactivated Gram-negative bacteria. Fresh PBLs or splenocytes (not transformed) may be used, if desired. A detailed description of the synthesis of the hybridomas, including the fusion protocol, [ELISAs]enzyme-linked immunosorbent assays (ELISAs) and hybrid screening procedure, exemplified in the following examples is disclosed in U.S. Serial No. 057,763, supra. The discussion of these procedures is incorporated herein by reference.

Please amend the paragraph at page 10, lines 16-28, to read as follows:

Following removal of non-adherent cells, a population of cells specifically enriched for the antigen used is obtained. These cells are transformed by EBV and cultured at approximately 10^3 cells per microtiter well using an irradiated lymphoblastoid feeder cell layer. Supernatants from the resulting lymphoblastoid cells are screened by ELISA against an E. coli Rc [LPS]lipopolysaccharide (LPS) and a Salmonella Re LPS. Cells that are positive for either Rc or Re lipid A LPS are expanded and fused to a 6-thioguanine-resistant mouse x human B cell fusion partner. If the mouse x human B cell fusion partner is used, hybrids are selected in ouabain and azaserine. Supernatatants from the Rc or Re positive hybrids are assayed by ELISA against a spectrum of Gram-negative baceria and purified Gram-negative bacterial LPSs. Cultures exhibiting a wide range of activity are chosen for in vivo LPS neutralizing activity. Many but not all antibodies so produced are of the IgM class and most demonstrate binding to a wide range of purified lipid A's or rough LPS's. The antibodies demonstrate binding to various smooth LPS's and to a range of clinical bacterial isolates by ELISA.

Please amend the paragraph at page 11, lines 16-21, to read as follows:

A one ml ampule of frozen D-234 stock (ATCC HB-9543) was thawed quickly in a 37°C water bath. The contents were aseptically added to 100 ml prewarmed, pregassed (95% air and 5% CO_2), serum-free HL-1 medium (Ventrex Labs, Portland, [Me]ME) supplemented with 0.1% Pluronic polyol F-68 and 8 mM L-glutamine in a 250 ml Erlenmyer flask with a loosely fitted plastic screw cap. The flask was placed in a humidified incubator (36.5°C, 90% relative humidity and 5% CO_2) and cultured with shaking at 100-120 [rpm]revolutions per minute (rpm).

In the Claims:

Please cancel claim 2 without prejudice to or disclaimer of the subject matter encompassed thereby.

Please amend claims 1, 3, and 6 to read as follows:

1. (amended) A method of determining the optimal level of product expression in animal cell culture wherein the concentration of a solute of interest in a culture medium composition for optimal product expression is different than the concentration of said solute in the culture medium composition determined for optimal cell growth, which method comprises:

e) growing the animal cell culture in a culture medium to determine optimal cell growth;

f) varying the concentration of the solute in the culture medium to a concentration above that optimal for cell growth, which concentration is effective to create an environment of solute stress on the cell culture as expressed by an inhibitory effect on cell growth or cell density of said cell culture;

g) monitoring the product expression [under the varying solute concentration conditions]as concentration of the solute is varied in the culture medium to determine optimal product expression; and

h) selecting the solute concentration that provides the optimal combination of cell growth and product expression, which allows for optimal productivity.

3. (amended) The method of claim [2]1 where said animal cell culture is a mammalian cell culture.

6. (amended) The method of claim [3]4, wherein said monoclonal antibodies are human or murine monoconal antibodies.